

**REMARKS**

**1. Election/Restriction**

In reviewing the final rejection, we became aware of certain errors/inconsistencies in the restriction requirement mailed March 15, 2005. It is important that these be corrected so that applicants may pursue the unelected invention(s) in divisional applications.

The March 15, 2005 restriction was as follows:

- I. Claims 1-5, drawn to a method of isolating pyruvate formate lyase (Pfl) defective lactic acid bacterium, classified in class 435, subclass, for example.
- II. Claims 6-11 and 27, drawn to a pyruvate formate lyase (Pfl) defective lactic acid bacterium and composition with this bacterium, classified in class 435, subclass, for example.
- III. Claims 12-24 and 28, drawn to a double defective mutant (Pfl and Ldh) lactic acid bacterium and composition with this bacterium, classified in class 435, subclass, for example
- IV. Claim(s) 25, drawn to a first method of using Pfl defective lactic acid bacterium for producing food, classified in class 435, subclass, for example.
- V. Claim(s) 26, drawn to a second method of using Pfl defective lactic acid bacterium for producing metabolite, classified in class 435, subclass, for example.

In response, we elected group II with traverse.

Applicant would like to file a divisional application with claims covering DN223 and DN224.

The present unelected group II, "doubly defective mutants", plainly covers DN223, see page 28, line 11.

Our first problem relates to DN224. DN224 is taught as

being Ldh defective but Pfl positive, see page 34, line 19, and is covered by claims 20-23. While claims 20-23 are included in group III, they are inconsistent with the definition of group III as being related to "doubly defective mutants".

Hence, the Examiner should either redefine group III as directed to Ldh defective mutants, whether or not Pfl is defective, or split III into IIIA (double defective mutants), and IIIB (Ldh defective, Pfl functional, mutants). Since group III was not elected, we think that the Examiner can still act on the merits of the present amendment, all we need is clarification of the status of claims 20-23 so we know whether to pursue them in the same divisional application as the doubly defective mutants.

The second problem relates to claim 24 and DN225. While claim 24 has been assigned to unelected group III, it is actually directed to Pfl- Ldh<sup>+</sup> mutants and thus would appear to have been properly classified in the elected group II. We believe that claim 24 was intended to cover DN225, see page 31, lines 16-18, and should have been examined in the instant application.

We cancelled claim 24 in good faith, accepting its classification into III. Since we are under final rejection, we cannot simply reinstate it as a new claim. However, we hope that the Examiner will consider adding a claim limited to DN225 by Examiner's amendment.

If not, then we will need to know whether we can rely on the classification of claim 24 into group III (i.e., can protect it by a divisional application) or whether we need to consider it to be a group II claim and pursue it either by filing an RCE or a continuation.

## 2. Prior Art Issues

2.1. The Examiner has conceded that claim 11, drawn to applicant's isolates DN221 and DN227 is free of prior art, and would be allowable if rewritten in independent form.

2.2. Likewise, the Examiner has conceded that claims 35-37 are free of the prior art, although they are rejected on non-art grounds.

2.3. Claims 6, 7, 9-10, 27, and 30-34 are rejected as anticipated by or obvious over Hugenholtz, Takahashi or Yamamoto in light of evidence from the ATCC catalogue.

With regard to the issue of anticipation by Yamamoto, the Examiner argues that we have not proven, by sequencing, the nature of the inactivation in our and Yamamoto's Pfl negative mutants. While that is true, the Examiner is arguing that Yamamoto inherently anticipates, and case law on inherency says that the inherent feature must be certain, not merely possible or probable. Thus, such case law suggests that it is the Examiner who bears the burden of proving that Yamamoto's mutants could not have been obtained by deletion of the entire Pfl gene.

2.4. However, we do not need to further address this issue, or the remaining prior art issues, at this time, because for this prosecution we have decided to accept limitation to the subject matter of DN221 and DN227 (and, if the Examiner is willing, DN225). This is without prejudice to pursuing the broader claims by continuation.

### 3. Definiteness Issues (OA pages 2-3)

The Examiner says that it is unclear which "characteristics" of the parent strains are included or excluded from the scope of claims 35-38.

This rejection is not well taken. The mutants of claim 35 (III) and (IV) are defined by a combination of a process limitation ("obtained by mutation....") and a functional limitation (at least one of four specifically recited characteristics (i)-(iv)). Even a purely functional limitation is not per se indefinite, see MPEP 2173.05(g). A product claim may also define the product in terms of the product in which it

is made, see MPEP 2173.05(p). Both functional and process limitations implicitly limit the structure of the product.

We have amended claim 35, paragraph (III) and (IV) to clarify that the mutant is still properly classified as a Lactococcus lactis. This term has an art-recognized significance in terms of biochemical, morphological and genetic characteristics. See, e.g., Bergey's Manual of Determinative Bacteriology (8<sup>th</sup> ed.), page 507, under the older name Streptococcus lactis (copy enclosed). For equivalency of L. lactis and S. lactis, see Wikipedia (copy enclosed).

The Examiner argues that mutant (III) must be drawn to a double mutant (Pfl-, Ldh-). That is a non sequitur. While the specification discloses mutating a Pfl- mutant to obtain a double mutant, the claim does not require that the mutant be a double mutant. It could, for example, be a mutant in which there are additional inactivating mutations in the Pfl- gene, thereby reducing the chance of reversion. It could instead be a mutant in which the additional mutations are phenotypically silent.

The Examiner then argues that by covering a double mutant, the claims are extending the scope of the claimed invention. There are numerous problems with this argument. First, while we agree that mutants (III) and (IV) include double mutants, they don't require them. Secondly, the specification discloses creation of double mutants, so such are within the scope of the disclosed invention. Finally, "extend" relative to what? Yes, clauses (III) and (IV) claim inventions not covered by clauses (I) and (II). A claim may contain subparagraphs which each cover different embodiments. Claim 35 is not dependent on any other claim, so there is no issue of it enlarging the scope of a base claim.

We suspect that the problem with coverage of double mutants is not a substantive one, but rather that the claim would then cover subject matter restricted out (as group III) by the 2005

restriction requirement.

Consequently, we have amended (III) and (IV) to exclude mutants which are Ldl-defective, thereby preserving the line of demarcation between elected group II and unelected group III.

The final sentence in the indefiniteness rejection, concerning DN227, seems to assume that mutants which have not been made cannot be definite. We think that is contrary to case law, cp. MPEP 2173.04, "Breadth is not indefiniteness". Note also that in Ex parte Jackson, 217 USPQ 804 (POBA 1982) a special nine member panel reversed the enablement rejection of a claim to a fermentation process reciting two deposited microorganisms "and mutations thereof".

We have amended claim 35 to specify that the mutants recited in (III) and (IV) are still properly classified as *Lactococcus lactis*, for what that conveys to the skilled worker concerning the biochemical, morphological and genetic characteristics of the bacterium. Thus, claim 35, (III) and (IV) now contain an explicit structural limitation, too.

#### 4. Written Description Issues (OA pages 3-4)

The Examiner asserts that "a mutant obtained by mutation of strain DN227" in claim 35 (i.e., mutant IV), and mutant (IV) in claims 36 and 38, have no support in the "as filed" specification. Note that the Examiner did not question support for mutant (III), i.e., mutants of DN221.

There is general support for mutating strains which are already Pfl-, see page 5, lines 18-26; page 12, lines 25-27; page 13, lines 3-32; page 18, lines 11-18; and page 35, lines 9-16.

DN227 is referred to at page 12, lines 17-21; page 25, lines 23-27, and page 39. Page 12, lines 7-21 imply that DN227 is Pfl defective.

We believe that such teaching, in combination with that of page 12, lines 25-27, provides adequate written description for

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use of DN227 as a parent strain for further mutation.

Respectfully submitted,

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Enclosure

-page 507 from Bergey's Manual  
-Wikipedia, "Lactococcus lactis"  
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IPC:lms  
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**BERGEY'S MANUAL OF  
DETERMINATIVE  
BACTERIOLOGY**

*Eighth Edition*

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1. Bacteriology—Classification. 2. Schizomycetes.

I. Bergey, David Hendricks, 1860-1937. II. Buchanan,  
Robert Earle, 1883-1973 ed. III. Gibbons, Norman  
Edwin, 1906- ed. IV. American Society for  
Microbiology. Bergey's manual of determinative  
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% tellurite or in 0.10% methylene  
produce an alpha-reaction on blood

ent for riboflavin or pyridoxal  
olysate medium. Folinic acid (or  
thymine) is required for growth  
eibel, 1967). Amino acid require-  
own.

C but response may be slow (48  
trains.  
of chickens characteristically, and  
m man, dogs and pigs.

as a marked, broad fermentation  
se fermentation, inability to hy-  
e, no growth in milk with 0.1%  
when sterilized separately and  
ly and the response to folinic acid  
is species.

*Streptococcus uberis* Diernhofer 1932,

*uber*, udder, teat; L. gen.n. *uberis*

from Seeley (1951).  
ring in pairs to chains of moderate

of this species has not been re-  
a third to a half of the strains.  
rious investigations react with  
um. To a lesser extent, reactions  
G and D antisera have been re-  
sky, 1969). These reactions ap-  
to similar type-specific antigens.  
9) was unable to obtain group E  
jection of *S. uberis* strains that  
ith group E antiserum.

ge in glucose broth 4.6-4.9. Acid  
uctose, maltose, lactose, sucrose,  
nitol, sorbitol and salicin. Most  
inulin but fail to ferment raffinose.  
se and melibiose not fermented.  
ited aerobically but not anaero-

ood agar may be characterized by  
r gamma-reaction.

mino acids, and usually seven B  
essential for growth in synthetic  
required.

perature 35-37 C.

and lips of cows (Cullen, 1966);  
lder tissue of cows with mastitis.  
identification scheme based on  
gical or serological procedures is  
similarity to the group D strep-  
that this species grows at 10 and  
lly survives 60 C for 30 min. Fur-  
olic acid requirement reflects a  
h *S. faecium*. However, the overall

nutritional and physiological pattern is de-  
finitively different and when considered in light  
of serological reactions, it is difficult to relate this  
species to the group D streptococci.

Reference strain: ATCC 19436; NCTC 3858.

20. *Streptococcus lactis* (Lister) Löhnis 1909,  
554. (*Bacterium lactis* Lister 1873, 408.)

*lac'tis*. L. n. *lac* milk; L. gen.n. *lactis* of milk.  
Ovoid cells elongated in direction of the chain;  
0.5-1.0  $\mu$ m in diameter. Mostly in pairs or short  
chains. Some cultures produce long chains.

The peptidoglycan is similar to that of *S.*  
*pyogenes* with the exception that the crossbridge  
consists of D-isoadipargine (Schleifer and Kandler,  
1967).

Lancefield's group N. The group antigenic  
determinant is a glycerol teichoic acid containing  
galactose phosphate (Elliott, 1963). It is not a  
wall constituent and it occurs intracellularly like  
the group D teichoic acid (Smith and Shattock,  
1964). The group N teichoic acid cross-reacts  
with certain type-specific antipneumococcal sera  
(Heidelberger and Elliott, 1966).

Many serological types are known.

Final pH range of 4.0-4.5 in glucose broth. Acid  
from glucose, maltose, lactose. Xylose, arabinose,  
sucrose, trehalose, mannitol and salicin may or  
may not be fermented. No acid from raffinose,  
inulin, glycerol or sorbitol. Tyrosine is not decar-  
boxylated. Some strains produce an antibiotic,  
nisin (Hirsch, 1951), that inhibits many Gram-  
positive organisms. Growth in media containing  
4.0 but not 6.5% sodium chloride. Growth initiated  
at pH 9.2 but not 9.6. Variations in the latter two  
characters occur. Grows in 0.3% methylene blue  
in milk. Some strains may metabolize leucine to  
produce 3-methylbutanal which gives a malty-  
flavor defect in dairy products (Jackson and Mor-  
gan, 1954).

On blood agar a weak alpha- or gamma-reaction  
is observed.

The nutrition of this species is comparatively  
complex. Generally, 4 or 5 of the B vitamins, 10  
to 13 amino acids (Niven, 1944) and acetate and  
oleate or lipoate (Collins *et al.*, 1950) are required  
for growth in synthetic media. Purines and pyrim-  
idines are not required but may be stimulatory.

Optimum temperature *ca.* 30 C. Some strains  
fail to grow at 41 C. No growth at 45 C.

A common contaminant in milk and dairy  
products (Stark and Sherman, 1935).

The G + C content of DNA ranges from 38.4-  
38.6 moles % ( $T_m$ ).

Intraspecies transformation does not occur.  
DNA from *S. lactis* can transform *S. sanguis*,  
strain Challis.

Reference strain: ATCC 19435; NCTC 6681.

20a. *Streptococcus lactis* subsp. *diacety-*  
*lactis* subsp. nov. (*Streptococcus diacetylactis* (*sic*)  
Matuszewski, Pijanowski and Supinska 1936, 23).  
*di.acety.lac'tis*. Reference to a *Streptococcus*  
*lactis* isolate that produces diacetyl.

This variety possesses the same characteristics  
as *S. lactis* except that it is capable of fermenting  
citrate (in conjunction with a fermentable car-  
bohydrate) with the production of carbon dioxide,  
acetoin and diacetyl.

21. *Streptococcus cremoris* Orla-Jensen 1919,  
132. (*Streptococcus hollandicus* Scholl 1891, 51;  
*Streptococcus lactis* B Ayers, Johnson and Mudge  
1924, 39.)

*cre.mo'ris*. L. n. *cremor* juice, cream; L. gen.n.  
*cremoris* of cream.

Spheres or ovoid cells elongated in direction of  
the chain; 0.6-1.0  $\mu$ m in diameter (often larger  
than *Streptococcus lactis*); form long chains, es-  
pecially in milk, but in some cultures predom-  
inantly as pairs.

The peptidoglycan is similar to that of *S. pyo-*  
*genes* except for differences in crossbridge com-  
pounds. Two types of crossbridges have been  
reported for *S. cremoris*: one is identical to *S.*  
*lactis* (D-isoadipargine); the other, a dipeptide  
consisting of L-alanyl-threonine (Schleifer and  
Kandler, 1967).

Lancefield's group N. The antigenic determinant  
is the same as that described for *S. lactis*. Many  
serological types are known to exist.

Final pH range of 4.0-4.5 in glucose broth. Acid  
produced from glucose and lactose. May or may  
not ferment trehalose and salicin. Rarely fer-  
ments maltose, sucrose, raffinose or mannitol.  
Arabinose, xylose, inulin, glycerol and sorbitol  
not fermented. In the presence of a fermentable  
sugar, some strains degrade citrate producing  
carbon dioxide, acetic acid and diacetyl. Some  
strains also produce antibiotic-like substances  
(Oxford, 1944).

On blood agar a weak alpha- or gamma-reaction  
is observed.

The nutritional characteristics closely parallel  
those of *S. lactis*.

Optimum temperature is about 30 C. No growth  
at 40 C.

Source: raw milk and milk products.

Differentiated from *S. lactis* which produces  
ammonia from arginine, generally grows in broth  
containing 4.0% sodium chloride, usually initiates  
growth in broth adjusted to pH 9.2 and grows in  
the presence of 0.3% methylene blue when added  
to milk. *S. cremoris* gives a negative reaction in  
these tests.

The G + C content of DNA ranges from 38-40  
moles % (method not stated).

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# Lactococcus lactis

From Wikipedia, the free encyclopedia

***Lactococcus lactis*** is a species of non-sporulating, non-motile, Gram-positive bacteria used extensively in the production of buttermilk and cheese.<sup>[1]</sup> *L. lactis* are cocci that group in pairs and short chains, typically 0.5 - 1.5 μm in length. When fermenting milk, *L. lactis* produce large quantities of lactic acid. Cultured in the laboratory, *L. lactis* colonies appear bright orange on nutrient agar.

## Cheese production

*L. lactis* subsp. *lactis* (formerly *Streptococcus lactis*<sup>[2]</sup>) is used in the early stages for the production of many cheeses including Brie, Camembert cheese, cheddar, Colby, Gruyère, Parmesan, and Roquefort.<sup>[3]</sup>

The use of *L. lactis* in dairy factories is not without issues. Bacteriophages specific to *L. lactis* cause significant economic losses each year by preventing the bacteria from fully metabolizing the milk substrate.<sup>[3]</sup> Several epidemiologic studies showed that the phages mainly responsible for these losses are from the species 936, c2 and P335.<sup>[4]</sup>

## References

1. ^ Madigan M, Martinko J (editors). (2005). *Brock Biology of Microorganisms*, 11th ed., Prentice Hall. ISBN 0-13-144329-1.

2. ^ Chopin MC, Chopin A, Rouault A, Galleron N (1989). "Insertion and amplification of foreign genes in the *Lactococcus lactis* subsp. *lactis* chromosome" (PDF). *Appl. Environ. Microbiol.* **55** (7): 1769-74. PMID 2504115.

3. ^ <sup>a</sup> <sup>b</sup> Coffey A, Ross RP (2002). "Bacteriophage-resistance systems in dairy starter strains: molecular analysis to application". *Antonie Van Leeuwenhoek* **82** (1–4): 303-21. DOI:10.1023/A:1020639717181. PMID 12369198.

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*Lactococcus lactis*

Scientific classification

Kingdom: Bacteria

Division: Firmicutes

Class: Bacilli

Order: Lactobacillales

Family: Streptococcaceae

Genus: *Lactococcus*

Species: ***L. lactis***

Binomial name

***Lactococcus lactis***

(Lister 1873)

Schleifer *et al.* 1986

Subspecies

*L. l. cremoris*

*L. l. hordniae*

*L. l. lactis*

*L. l. lactis* bv. *diacetylactis*

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